2. Prokocimer, M., Davidovich, M., Nissim-Rafinia, M., Wiesel-Motiuk, N., Bar, D., Barkan, R., Meshorer, E., and Gruenbaum, Y. (2009). Nuclear lamins: key regulators of nuclear structure and activities. J. Cell Mol. Med. *13*, 1059–1085.
3. Chen, C.-Y., Chi, Y.-H., Mutalif, R.A., Starost, M.F., Myers, T.G., Anderson, S.A., Stewart, C.L., and Jeang, K.-T. (2012). Accumulation of the inner nuclear envelope protein sun1 is pathogenic in progeric and dystrophic laminopathies. Cell *149*, 565–577.
4. Lei, K., Zhu, X., Xu, R., Shao, C., Xu, T., Zhuang, Y., and Han, M. (2012). Inner nuclear envelope proteins SUN1 and SUN2 play a prominent role in the DNA damage response. Curr. Biol. *22*, 1609–1615.
5. Starr, D.A., and Fridolfsson, H.N. (2010). Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. Annu. Rev. Cell Dev. Biol. *26*, 421–444.
6. Sosa, B.A., Rothballer, A., Kutay, U., and Schwartz, T.U. (2012). LINC complexes form by binding of three KASH peptides to domain interfaces of trimeric SUN proteins. Cell *149*, 1035–1047.
7. Zhang, X., Lei, K., Yuan, X., Wu, X., Zhuang, Y., Xu, T., Xu, R., and Han, M. (2009). SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. Neuron *64*, 173–187.
8. Ding, X., Xu, R., Yu, J., Xu, T., Zhuang, Y., and Han, M. (2007). SUN1 is required for telomere attachment to nuclear envelope and gametogenesis in mice. Dev. Cell *12*, 863–872.
9. Yu, J., Lei, K., Zhou, M., Craft, C.M., Xu, G., Xu, T., Zhuang, Y., Xu, R., and Han, M. (2011). KASH protein Syne-2/Nesprin-2 and SUN proteins SUN1/2 mediate nuclear migration during mammalian retinal development. Hum. Mol. Genet. *20*, 1061–1073.
10. Haque, F., Mazzeo, D., Patel, J.T., Smallwood, D.T., Ellis, J.A., Shannahan, C.M., and Shackleton, S. (2010). Mammalian SUN protein networks at the inner nuclear membrane and their role in laminopathy disease processes. J. Biol. Chem. *285*, 3487–3498.
11. Kandert, S., Luke, Y., Kleinhenz, T., Neumann, S., Lu, W., Jaeger, V.M., Munck, M., Wehnert, M., Muller, C.R., Zhou, Z., *et al*. (2007). Nesprin-2 giant safeguards nuclear envelope architecture in LMNA S143F progeria cells. Hum. Mol. Genet. *16*, 2944–2959.
12. Zhang, Q., Bethmann, C., Worth, N.F., Davies, J.D., Wasner, C., Feuer, A., Ragnauth, C.D., Yi, Q., Mellad, J.A., Warren, D.T., *et al*. (2007). Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. Hum. Mol. Genet. *16*, 2816–2833.
13. Puckelwartz, M.J., Kessler, E., Zhang, Y., Hodzic, D., Randles, K.N., Morris, G., Earley, J.U., Hadhazy, M., Holaska, J.M., Mewborn, S.K., *et al*. (2009). Disruption of nesprin-1 produces an Emery Dreifuss muscular dystrophy-like phenotype in mice. Hum. Mol. Genet. *18*, 607–620.
14. Oza, P., Jaspersen, S.L., Miele, A., Dekker, J., and Peterson, C.L. (2009). Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. Genes Dev. *23*, 912–927.
15. Gonzalez-Suarez, I., and Gonzalo, S. (2010). Nurturing the genome: A-type lamins preserve genomic stability. Nucleus *1*, 129–135.
16. Liu, B., Wang, J., Chan, K.M., Tjia, W.M., Deng, W., Guan, X., Huang, J.-D., Li, K.M., Chau, P.Y., Chen, D.J., *et al*. (2005). Genomic instability in laminopathy-based premature aging. Nat. Med. *11*, 780–785.

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Pollination: The Price of Attraction

Nectar is the major currency bringing together plants and pollinators; yet the costs and benefits of nectar production remain poorly understood. A low nectar line developed in *Petunia* offers an innovative approach to this problem and may offer clues to why some plants cheat and secure pollination via deception.

Michael R. Whitehead, Ryan D. Phillips, and Rod Peakall

While plants use a diverse range of visual and olfactory cues to advertise to pollinators [1], nectar is the major currency by which plants sustain repeat pollinator visitation [2]. So what are the costs and benefits of nectar production? How can plants optimise nectar production within a complex fitness landscape of competing interests, and how do genetic and physiological constraints on seed set, pollinator behaviour and community context influence the outcome?

Manipulative experiments are crucial for addressing these questions. These have shown that plants can enhance seed set and pollen removal by increasing nectar production [3–5] but also indicate that nectar can incur a fitness cost to the plant both energetically [6], and through increased self pollination [7–9] (Figure 1). Despite their simple elegance, such experiments are labour intensive, short rather than long term, and can damage the flower. Furthermore, simultaneously measuring lifetime reproductive fitness as a function of nectar production is difficult [2], perhaps even impossible.

As reported in this issue of *Current Biology*, Brandenburg *et al.* [10] have employed an innovative complement to experimental manipulation that promises new clues about the cost of nectar production. They exploited the model system *Petunia* to develop an introgression line, called F25, with the desired trait of low nectar volume. This line was constructed by performing an initial hybrid cross between the interfertile low volume nectar-producing *P. integrifolia* and the high volume nectar-producing *P. axillaris*, followed by three successive backcrosses to *P. axillaris*. For subsequent laboratory experiments, F25 and control lines were vegetatively propagated.

For introgression lines to be informative, they need to be similar in all respects to control lines, but for the trait of interest. Brandenburg *et al.* [10] confirmed that flower color, shape and size, as well as pollen and ovule production in F25 were indistinguishable from the control parent. Furthermore, 64 out of 65 diagnostic genetic markers matched *P. axillaris.* As planned, nectar volume was reduced in F25, being on average 30% of the nectar volume of the controls.

In the laboratory, naive hawkmoth pollinators did not discriminate between F25 and control plants. However, probing time at flowers was significantly lower at F25 flowers. This behavioural difference translated to reduced seed set, confirming a fitness cost of reduced nectar production. A critical additional finding was that hand-pollinated seed set was significantly higher in F25, suggesting that the energetic savings on nectar production might be re-invested by the plant to enhance fitness. This exciting finding supports the expectation that nectar production has important lifetime fitness consequences for plants [6].

The development of line F25 and these initial laboratory experiments are but the first step. Demonstration of its full potential awaits its

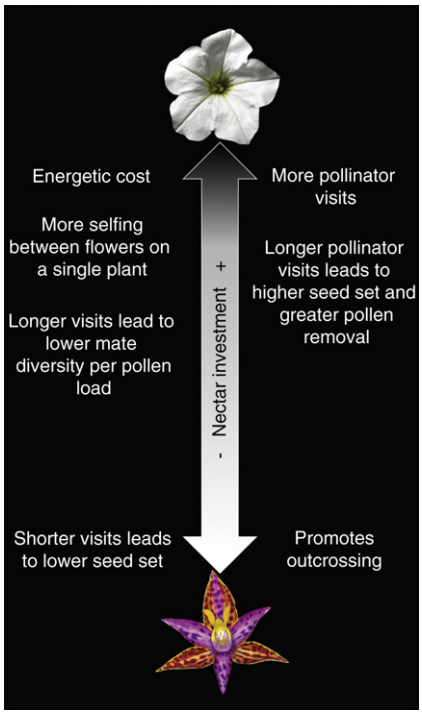

Figure 1. Trade-offs associated with floral rewards.

The spectrum of nectar investment strategies observed in nature is associated with a complex trade-off between cost (left) and benefit (right). For example, hermaphroditic flowers producing high nectar flowers that encourage pollinators to linger can increase seed set, but potentially at the costs of energy, mate diversity and outcrossing. Pictured are the high nectar-producing *Petunia axillaris* (top) and rewardless food-deceptive orchid *Thelymitra pulcherrima* (bottom).

translocation to the field in the native range of *Petunia*. Future experiments employing arrays of F25 and control lines may allow for the first time a comprehensive assessment of the lifetime fitness consequences of nectar production. Also of particular interest in future field experiments will be an exploration of why the other hybrid parent, the purple-flowered, bee-pollinated *P. integrifolia*, is characterised by low nectar volumes.

Introgression lines offer a complementary approach to the recent use of genetic modification [11,12] for teasing apart the complex interactions between plants and pollinators. When traits of interest are under simple genetic control, and candidate genes are known, genetic modification may be the more efficient and flexible approach. However, introgression lines will be the best option when the target is a quantitative trait. The use of introgression lines also avoids the regulatory complexity of working with genetically modified organisms, particularly in the field. In the case of *Petunia*, with its natural range in South America, there are severe restrictions on the use of transgenic lines in the field.

The development of introgression and transgenic lines can have unintended consequences. For example, in line F25, floral scent production was also enhanced. As scent was saturated under the laboratory conditions, this may be of little consequence in the pollinator choice experiments of Brandenburg *et al.* [10]; however, it may pose challenges for disentangling the role of nectar and odour in field experiments.

On the other hand, such unexpected consequences may be turned to advantage. Floral odour often plays a critical role in pollinator attraction [1,13–15]. Line F25 with its enhanced floral odour production may offer unique opportunities for testing the role of odour variation in pollinator attraction and specificity. What is more, while gene silencing approaches can lower odour production [11], enhancing odour production is likely to be more difficult and best achieved via introgression lines.

Rewardless plants are one group of special interest for exploring the ecological and evolutionary costs of nectar production. The orchids in particular offer unique research opportunities. Several thousand species employ a diversity of deceptive pollination strategies [16–18] and confirm that deception can be a stable evolutionary strategy. The diversity of life histories in these orchids suggests very different combinations of evolutionary processes may favour the evolution of reward over deception, and vice versa, depending on the ecological context.

Presently the favoured hypothesis for the evolution and maintenance of rewardless flowers is inbreeding avoidance [16]. To test this hypothesis, and evolutionary questions in plants more generally [19], it is essential to move beyond mere seed set as the measure of fitness and assess both male and female components of fitness, and the genetic quality of pollination. This remains an outstanding requirement for future experiments involving introgression line F25 in *Petunia*.

Without question, the strategic use of introgression and transgenic lines opens up exciting new lines of scientific enquiry. Unfortunately these approaches will be challenging to implement in non-model organisms. Nonetheless, one feature of both methods that could be employed more widely is the clonal propagation of lines with target traits of interest [20]. When combined with creative manipulative experiments in the wild, these tools hold promise for answering the outstanding question of the cost of nectar production and may also offer insights into why some plants are able to cheat the system and secure pollination via deception.

References

1. Raguso, R.A. (2004). Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. Curr. Opin. Plant Biol. *7*, 434–440.
2. Brandenburg, A., Dell'Olivo, A., Bshary, R., and Kuhlemeier, C. (2009). The sweetest thing: advances in nectar research. Curr. Opin. Plant Biol. *12*, 486–490.
3. Zimmerman, M. (1983). Plant reproduction and optimal foraging: experimental nectar manipulations in *Delphinium nelsonii*. Oikos *41*, 57–63.
4. Ackerman, J.D., Rodriguez-Robles, J.A., and Melendez, E.J. (1994). A meager nectar offering by an epiphytic orchid is better than nothing. Biotropica *26*, 44–49.
5. Mitchell, R.J. (1993). Adaptive significance of *Ipomopsis aggregata* nectar production: observation and experiment in the field. Evolution *47*, 25–35.
6. Pyke, G.H. (1991). What does it cost a plant to produce floral nectar? Nature *350*, 58–59.
7. Jersáková, J., and Johnson, S.D. (2007). Lack of reward promotes outcrossing: nectar supplementation experiment with *Disa pulchra* (Orchidaceae). S. Afr. J. Bot. *73*, 328.
8. Jersáková, J., Johnson, S.D., Kindlmann, P., and Pupin, A.-C. (2008). Effect of nectar supplementation on male and female components of pollination success in the deceptive orchid *Dactylorhiza sambucina*. Acta Oecologica *33*, 300–306.
9. Johnson, S.D., Peter, C.I., and Agren, J. (2004). The effects of nectar addition on pollen removal and geitonogamy in the non-rewarding orchid *Anacamptis morio*. Proc. R. Soc. Lond. B *271*, 803–809.
10. Brandenburg, A., Kuhlemeier, C., and Bshary, R. (2012). Hawkmoth pollinators decrease seed set of a low-nectar *Petunia axillaris* line through reduced probing time. Curr. Biol. *22*, 1635–1639.
11. Kessler, D., Gase, K., and Baldwin, I.T. (2008). Field experiments with transformed plants reveal the sense of floral scents. Science *321*, 1200–1202.
12. Kessler, D., and Baldwin, I.T. (2011). Back to the past for pollination biology. Curr. Opin. Plant Biol. *14*, 429–434.
13. Schiestl, F.P., Peakall, R., Mant, J.G., Ibarra, F., Schulz, C., Franke, S., and Francke, W. (2003). The chemistry of sexual deception in an orchid-wasp pollination system. Science *302*, 437–438.

14. Whitehead, M.R., and Peakall, R. (2009). Integrating floral scent, pollination ecology and population genetics. Funct. Ecol. *23*, 863–874.
15. Klahre, U., Gurba, A., Hermann, K., Saxenhofer, M., Bossolini, E., Guerin, Patrick M., and Kuhlemeier, C. (2011). Pollinator choice in *Petunia* depends on two major genetic loci for floral scent production. Curr. Biol. *21*, 730–739.
16. Jersáková, J., Johnson, S.D., and Kindlmann, P. (2006). Mechanisms and evolution of deceptive pollination in orchids. Biol. Rev. *81*, 219–235.
17. Schiestl, F.P. (2005). On the success of a swindle: pollination by deception in orchids. Naturwissenschaften *92*, 255–264.
18. Cozzolino, S., and Widmer, A. (2005). Orchid diversity: an evolutionary consequence of deception? Trends Ecol. Evol. *20*, 487–494.
19. Karron, J.D., Ivey, C.T., Mitchell, R.J., Whitehead, M.R., Peakall, R., and Case, A.L. (2012). New perspectives on the evolution of plant mating systems. Ann. Bot. *109*, 493–503.
20. Mitchell, R.J. (2004). Heritability of nectar traits: why do we know so little? Ecology *85*, 1527–1533.

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Algal Biogeography: Metagenomics Shows Distribution of a Picoplanktonic Pelagophyte

How can we determine the distribution of uncultured marine microorganisms? Targeted metagenomics has provided the complete chloroplast genome sequence, and the distribution, for a picoplanktonic pelagophyte alga.

John A. Raven

Determining the diversity, and the functional significance, of marine micro-organisms has been hampered by our inability to culture and hence characterise the majority of the microorganisms which have been isolated. Metagenomic studies using the innovative technique of at-sea fluorescence activated cell sorting have allowed Worden *et al.* [1], as reported in this issue of *Current Biology*, to construct a complete chloroplast genome sequence for a eukaryotic picoeukaryote from the Gulf Stream Current. This technique generates reference genome information from abundant natural populations without the need for culturing. The organism containing the sequenced chloroplast genome is a member of the genus *Pelagomonas*, based on the 100% sequence similarity of the rubisco (ribulose bisphosphate carboxylase-oxygenase) large subunit gene (rbcL) from the Gulf Stream organism with the partial sequences from cultured *Pelagomonas calceolate* [1]. Despite this complete sequence similarity, Worden *et al.* [1] opted for the cautious conclusion that the uncultured population is "wild *Pelagomonas*" rather than the plausible view that the organism from the Gulf Stream is *Pelagomonas calceolate*, a member of the class Pelagophyceae.

What is the Pelagophyceae? The class Pelagophyceae was erected in 1997 to contain a number of small-celled heterokontophyte algae united by molecular genetic, ultrastructural and (to a lesser extent) photosynthetic pigments [2,3]. They are all marine and mainly planktonic, and include the organisms which have caused brown tides in the coastal waters off the east coast of the USA (*Aureococcus anophagefferens*) and the Gulf of Mexico (*Aureaumbra lagunensis*), as well as the open ocean flagellate *Pelagomonas calceolata* and coccoid *Pelagococcus subviridis* [2,3]. They have been subject to significant ecophysiological and genomic analysis [4,5].

The work of Worden *et al.* [1] provides an excellent example of a novel method of estimating the distribution of an uncultured marine microorganism. Worden *et al.* [1] point out that other methods of estimating the (quantitative) occurrence of pelagophyceans are less precise; examples are the use of primer-based molecular genetic and photosynthetic pigment analyses. Radioactive inorganic carbon labelling of total cell carbon and of pigments have been used to estimate the contribution of taxonomic groups of phytoplankton (identified by their pigment composition) to net primary productivity in the northwestern Mediterranean [6]. The allocation of the total phytoplankton chlorophyll-a among taxa used Chemtax, an algorithm based on the mean ratios of photosynthetic pigments in each of a range of algal classes that allocates biomass to these classes based on their contribution to the overall pigmentation in a phytoplankton assemblage. For allocation to pelagophyceans it was assumed that light-harvesting carotenoid 19'-butanoyloxofucoxanthin was unique to, and ubiquitous within, the Pelagophyceae. The former assumption may not be true [7], nor may the latter [2]. Granted the assumptions made, the Pelagophyceae contributed 4% to net primary production and 4.7% to the chlorophyll-a content of the phytoplankton from 4–8 metres depth. 81% of the Pelagophyceae were less than 5 μm in equivalent spherical diameter [6]. Under the conditions of the observations, the specific growth rate of the pelagophyceans was 0.87 units cell mass increase per unit cell mass per day, with an equal specific rate of grazing (i.e., no change in population size) [6]. The pelagophycean growth rate was close to that of the phytoplankton community as a whole (i.e., 0.89 per day) [6].

Studies of the (mainly eukaryotic) phytoplankton with effective spherical diameters of less than 3 μm in the open ocean of the Arabian Sea using PCR of 16S rRNA were biased toward eukaryotic plastid sequences rather than cyanobacterial sequences [8]. Granted the assumptions made (see [1]), pelagophyceans were 0–8% of the total at the depths examined (i.e., 10 and 35 m at Station 1; 44 and 64 m at Station 2) [8]. Using FISH technology [9] it was found that the Pelagophyceae contribute 2 and 10% of eukaryotic phytoplankton biomass with effective cell diameter less than and more than